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13. ABSTRACT *(Maximum 200 Words)*
Women who inherit a BRCA1 or BRCA2 mutation have an 80-90% chance of developing breast cancer. Gene targeting techniques were used to create a Brca2-deficient mouse. A portion of exon 10 was replaced with the Neo gene resulting in premature truncation of the protein product. Examination of normal mammary ductal development in $129^{(+/+)}$ and $129^{(+/Brca2-)}$ mice revealed phenotypic differences between the two genotypic classes. $129^{(+/+)}$ and $129^{(+/Brca2-)}$ mice and several inbred mouse strains were used to study the effect of genetic background on radiation induced mammary tumor induction. BALB/c and SWR mice are susceptible to radiation induced mammary tumorigenesis and C57BL/6, FVB/N and C3H mice are relatively resistant. While FVB/N mice do not develop tumors, radiation treatment has a dramatic effect on ductal morphogenesis. To date, no mammary tumors have been observed in the $129^{(+/+)}$ and $129^{(+/Brca2-)}$ mice. Brca2-deficient mice were crossed to p53 mutant mice to generate four genotypic classes of offspring. A study is in progress to assess the consequences of harboring mutation in the two tumor suppressor genes with and without radiation exposure. The mammary tumor susceptible BALB/c and resistant C57BL/6 mouse strains being used to make mice congenic for the Brca2 mutation.

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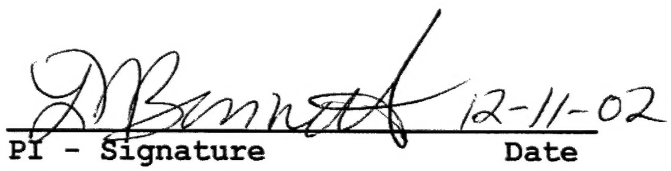

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Introduction

Approximately 180,000 women will be diagnosed with breast cancer this year and 43,000 women will die from the disease. It is the most commonly diagnosed cancer and the second leading cause of cancer deaths among women. Approximately 7% of breast cancers are attributed to the inheritance of BRCA mutations. Women who inherit a mutated copy of the BRCA2 gene have a 28% chance of developing breast cancer before the age of 50 and a lifetime risk that has been determined to be as high as 85%. In order to better understand the consequences of inheriting an alteration in the BRCA2 gene I proposed to develop a mouse model using gene targeting technology. $129^{(+/Brca2-)}$ deficient mice were created with a targeted mutation in exon 10 of the endogenous mouse Brca2 gene. The study objectives for the use of these mice were to: (a) assess differences in normal and neoplastic growth control in mice carrying one or two defective copies of the BRCA2 gene, (b) determine the cancer risks of radiation exposure in mice with BRCA2 defects, and (c) study the effect of carrying defects in two tumor suppressor genes by mating BRCA2-deficient mice with transgenic mice that carry a mutant copy (Ala135Val) of the p53 tumor suppressor gene. These studies were designed to understand potential gene-environment and gene-gene interactions. It was of particular interest to study germline Brca2 mutations in association with radiation, a known breast carcinogen, and a mutated p53 gene, which when inherited also predisposes women to breast cancer development.

Body

Specific Aim 1:

Development of a Brca-2 deficient mouse

As described in the 1999 Annual Report, this aim of the proposal has been completed. As described in the 2000 Annual Report these mice have been used to address the objectives proposed in the originally submitted grant application, as well as other studies.

Specific Aim 2:

Information Described in the 2001 Annual Report

1. It was determined that Brca2-null mice on the BALB/c genetic background die at embryonic day 10.5, two days later than on the 129/SvEv background
2. The two-year study to evaluate the response of several inbred mouse strains to mammary tumor induction by low-level radiation has been completed. The tissues from C57BL/6NCI, BALB/cJ, C3H/HeNCI, SWR/J, and FVB/N inbred mouse strains are being evaluated.
3. The two year final sacrifices began in the fall of 1999 and there were less than 10 mice left to be sacrificed. Gross pathology had not been observed that distinguished mice with and without a Brca2 mutation. Conclusions have been made on the pathology and diagnosis has been completed. Manuscript is being prepared for submission.

Current and Extended Results

Animals

All the animals that were used in these experiments have been euthanized. There are no living animals left to be euthanized for this study. The animals are represented by tissues and mammary gland whole mounts preserved for microscopic and macroscopic analysis.

Radiation induced mammary tumorigenesis in inbred mouse strains

The data for this portion of the aim have been collected. An application for the transfer of the remaining funds for this proposal has been submitted to the DOD in addition for a request for a one-year no-cost extension. A manuscript is being prepared describing the effects of whole body radiation in the C3H/HeNCI, C57BL/6NCI, FVB/N, BALB/cJ and SWR/J inbred mouse strains. (attached)

Does radiation exposure of $129^{(+/Brca2-)}$ mice increase their risk for mammary tumor induction?

Some slides have been prepared and are waiting pathological evaluation and diagnosis by John Seely and Barb Davis (NIEHS). From there we will be able to perform statistical analyses and determine if there are any differences among the treated and control groups with and without a Brca2 mutation. At the current time there is no evidence for increased risk to the $129^{(+/Brca2-)}$ mice.

Specific Aim 3:

Reported in the 2000 and 2001 Annual Reports

1. To date, we have not observed an increased incidence of mammary tumors in the FVB129F1$^{(+/Brca2-)}$, FVB129F1$^{(p53mut/Brca2-)}$, or FVB129F1$^{(p53mut/+)}$ compared to one another or the wild type FVB129F1$^{(+/+)}$ littermates. In addition, no other gross pathology, apparent at necropsy, distinguishes the genotypic classes. Partial or full necropsies were performed on the mice that became moribund during the course of the experiment. All the tissues from the mice are being processed for routine histology

Current Status

The fourth abdominal mammary glands have been stained and mounted to slides for morphologic assessment.

Specific Aim 4:

Information Described in the 2000 and 2001 Annual Reports

The Brca2 mutation was made congenic on the BALB/c genetic background. BALB/c mice are susceptible to radiation-induced mammary tumorigenesis and radiation-induced changes in mammary ductal morphology after relatively low exposures.

Summary:

The work proposed in this grant application is completed. We have data on the many animals that were included in Specific Aims 2 and 3. However, because there has been no funding for this proposed work since early September 2000 final or re-analyses of tissue has not been completed. As a result data have not been fully collected or analyzed. The study of the inbred mouse strains has been completed without being funded. The remainder of the work on this grant may be completed depending on the availability of personal time. There are no funds to cover any additional work.

Key Research Accomplishments

The funding for this grant was stopped in September 2000. Dr. Michelle Bennett, in keeping with the philosophy of the DOD's postdoctoral training grant mechanism, secured an independent position in which she is currently trying to establish herself as an independent investigator in the field of Breast Cancer Research. Upon her move to Lawrence Livermore National Laboratory (LLNL) to begin her new position she was informed by the USAMRMC she could apply to have the funds remaining from her postdoctoral training grant transferred to LLNL. Dr. Bennett completed and submitted the required paperwork in December of 2000.

At this time Dr. Bennett was advised to submit a request for a no-cost extension because the process of review for the transfer of funds might take several months. Dr. Bennett submitted a request for a no-cost extension for this study in December 2000.

In April 2001 Dr. Bennett inquired about the status of the potential transfer of funds and was told that all the required documentation was in the hands of the DOD except for a copy of the current Institutional Animal Care and Use Committee approved Animal Protocol for the funded study. Dr. Bennett explained that she had submitted the Approved Animal Protocol that covered the use of all animals in the study which originated at NIEHS, NIH where she did her postdoctoral training. All Animals in the study were housed and sacrificed at NIEHS. All animals were sacrificed before Dr. Bennett's move to LLNL. As a result, there were no animals for which to prepare an animal protocol for the Institutional Animal Care and Use Committee at LLNL. Dr. Bennett did not seek approval for the use of the animals that were used in this study because there were no live animals left in the study.

Very little has been done for this study in the last 2 years. The final analyses, data collection and enclosed manuscript have been completed on Dr. Bennett's personal time.

List of Reportable Outcomes

Papers

Brca2-Null Embryonic Survival is Prolonged on the BALB/c Genetic Background. L. Michelle Bennett, Kimberly A. McAllister, Pamela E. Blackshear, Jason Malphurs, Gina Goulding, N. Keith Collins, Toni Ward, Donna O. Bunch, Edward M. Eddy, Barbara J. Davis, and Roger W. Wiseman. Molecular Carcinogenesis, 28:174-183, 2000.

Mice heterozygous for a *Brca1* or *Brca2* mutation display distinct mammary gland and ovarian phenotypes in response to diethylstilbestrol. L. Michelle Bennett, Kimberly A. McAllister, Jason Malphurs, Toni Ward, N. Keith Collins, John C. Seely, Lori C. Gowen, Beverly H. Koller, Barbara J. Davis, and Roger W. Wiseman. Cancer Research, 60: 3461-3469, 2000.

In addition a paper that used the C57BL/6 strain congenic for the Brca2 mutation was published: Mammary Tumor Induction and Premature Ovarian Failure in Apc^{Min} Mice are Not Enhanced by *Brca2* Deficiency. Bennett, L.M., McAllister, K.A., Ward, T., Malphurs, J., Collins, N.K., Seely, J.C., Davis, B.J., and Wiseman, R.W.. *Toxicologic Pathology*, 29: 117-125, 2001.

Funding Applications

Dr. Bennett was funded for one grant in which in which data obtained from this funded study were included in the preliminary data section.

1. Grant application to the California Breast Cancer Research Program January 11, 2001. Dr. Bennett was funded for this project.

Conclusions

A mouse that is heterozygous for a Brca2 mutation has been developed on the BALB/c, 129 and C57BL/6 genetic backgrounds. Strain 129 mice that inherit a mutated copy of Brca2 do not appear to be predisposed to an increased incidence of spontaneous or radiation-induced mammary tumors compared to wild type controls. When two copies of the Brca2 mutation are inherited on the129 or a mixed 129 and C57BL/6 background the embryos die at d8.5 gestation. BALB/c mice that have inherited two mutant copies of Brca2 are also embryonic lethal. However, we have determined that the day of death is prolonged to d10.5 on the BALB/c background.

References

Brca2-Null Embryonic Survival is Prolonged on the BALB/c Genetic Background. L. Michelle Bennett, Kimberly A. McAllister, Pamela E. Blackshear, Jason Malphurs, Gina Goulding, N. Keith Collins, Toni Ward, Donna O. Bunch, Edward M. Eddy, Barbara J. Davis, and Roger W. Wiseman. *Molecular Carcinogenesis*, 28:174-183, 2000.

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Mammary Tumor Induction and Premature Ovarian Failure in Apc^{Min} Mice are Not Enhanced by *Brca2* Deficiency. L. Michelle Bennett, Kimberly A. McAllister, Toni Ward, Jason Malphurs, Keith Collins, John C. Seely, Barbara J. Davis, and Roger W. Wiseman. *Toxicologic Pathology*, 29: 117-125, 2001.

Appendix

DRAFT Document: Radiation-induced carcinogenesis and altered mammary ductal morphology in inbred mouse strains

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Radiation-induced carcinogenesis and altered mammary ductal morphology in inbred mouse strains

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Abstract

Numerous mouse models are being developed on an array of inbred genetic backgrounds to study the impact of genetic changes on normal and neoplastic development. We designed a study to compare the patterns of mammary ductal morphology and tumor susceptibility among inbred mouse strains commonly used for the development of transgenic and gene-deficient models as well as for carcinogenesis studies. The inbred strains BALB/cJ, SWR/J, FVB/N, C57BL/6N, and C3H/HeN mice were left untreated or exposed to a single 0.3 Gy dose at 5 weeks of age. Mice were sacrificed at 2, 3, 6, 9, and 12 months of age for the evaluation of mammary ductal morphology, and at 24 months or when moribund for tumor development. The mammary ductal branching pattern and tumor susceptibility characteristics were unique for each inbred mouse strain examined. BALB/c and SWR were sensitive to radiation-induced mammary tumorigenesis with incidences of 27% and 58%, respectively. The mammary glands from radiation-exposed BALB/c mice were characterized by complex branching, ductal dilation, and occasional galactocele formation and were associated with mammary tumor sensitivity. FVB mice responded to radiation exposure with a dramatic inhibition of mammary ductal side-branching and alveolar bud formation and did not display a change in tumor susceptibility. C3H was highly sensitive to radiation-induced ovarian tumor development with an incidence of 95%. Genetically modified mice can provide useful models for the effects of environmental exposures, lifestyle, or tumor prevention strategies on human cancer risk. Their use as models for mammary and ovarian carcinogenesis models is enhanced by understanding strain-specific characteristics of normal and neoplastic changes in mammary ductal development and tumorigenesis.

Introduction

Genetically modified mouse models for human cancer development are being developed at an extraordinary pace. These mouse models are being used to study tumor progression, metastesis, and prevention, as well as to define the mechanism(s) of action of the particular gene or molecular pathways being investigated. It is well established that inbred mouse strains have different susceptibilities to spontaneous and carcinogen-induced tumorigenesis. Variation among inbred mouse stains provides a powerful approach to study environmental and genetic factors that may modify the risk of carcinogenesis in genetically modified animals as well as to identify appropriate inbred strain backgrounds for the study of genetic alterations.

Radiation is one of the most studied human carcinogens and is one of the few known environmental risk factors for breast cancer (1). Japanese atomic bomb survivors, women who were treated for Hodgkin's disease in their youth, and patients who underwent fluoroscopic examinations for tuberculosis and other indications have an elevated risk of breast cancer (2-5). The increase in breast cancer risk remains constant for at least several decades beginning five to ten years after radiation exposure (3). Thus, radiation induces damage that can persist until a permissive microenvironment is established and tumor promotion and progression can proceed. The biologic effects of radiation exposure include cell cycle arrest or apoptosis, gene expression changes, alterations in microenvironment, and the induction of chromosomal breakage that can cause duplications, deletions, or translocations (6-11).

Mice have been used to evaluate radiation exposure effects and have been used for risk assessment and extrapolation to humans (11-13). Mouse studies clearly demonstrate that genetic background is a critical variable in survival and tumorigenesis after radiation exposure. Inbred mouse strains display different mean survival times after exposure to daily doses of radiation

(11,13). For example, female 129/J and SJL/J mice survive longer and are among the more resistant, whereas the BALB/c, SWR, and C3H strains are more sensitive (11,12). Radiation-induced tumorigenesis has been examined in several radiation-sensitive and -resistant inbred mouse strains (12,14). BALB/c mice are relatively susceptible to radiation-induced mammary and lung tumors, C3Hf/Bd mice are susceptible to radiation-induced mammary, ovarian, and liver tumors, whereas C57BL/6 mice are relatively resistant to radiation-induced tumorigenesis (12,14).

There is strong precedent for the use of inbred mouse strains that are more and less susceptible to tumor induction to identify both tumor susceptibility genes as well as genetic modifiers (15,16). For example, the liver tumor sensitive C3H and DBA and several resistant strains including C57BL/6 were used to map the hepatocarcinogen sensitivity loci (17,18). Crosses between ApcMin and AKR mice led to the identification of the intestinal tumor modifier Mom-1 (15) and crosses to other inbred mouse strains revealed mammary and ovarian tumor modifiers in Apc mutant mouse models (19,20).

Knowledge about the responses of inbred mouse strains to radiation exposure is critical in the context of understanding the genetics of susceptibility, interactions between genetic background and environmental exposure, and inbred strain background contributions in genetically engineered mouse models. Radiation-induced genetic damage is of particular interest since several cancer susceptibility genes are involved in DNA repair or are influenced by radiation exposure (21). In this report, radiation-induced alterations in mammary ductal branching patterns and tumorigenesis were examined in five inbred mouse strains.

The consequences of low doses of radiation exposure on mammary gland development were inbred strain specific. The normal pattern of ductal branching and tumor susceptibility characteristics in untreated and treated mice are unique for each inbred mouse strain examined.

Materials and Methods

Mice

Female BALB/cJ (BALB/c) and SWR/J (SWR) mice were obtained from the Jackson Laboratory (Bar Harbor, ME). C3H/HeN^{MMTV}-NCI (C3H), C57BL/6NCI (C57BL/6), and FVB/NCI (FVB) mice were purchased from Charles River Laboratory (Raleigh, NC). Mice were housed in plastic cages, up to four per cage, with a 12-h on-off light cycle, and fed NIH 31 diet (18% protein, 4% fat, 5% crude fiber; Zeigler Bros., Inc., Gardners, PA) and water *ad libitum*.

Radiation Treatment

Mice of each inbred strain were assigned to a treated group of 50 and a control group of 40 mice. The treated group was exposed to a single radiation treatment of 0.3 Gy with a Model 431 Cs-137 Irradiator (J.L. Shepherd and Assoc., Glendale, CA) at 35 ± 2 days of age and an exposure rate of 9.5 Rad/min. During the exposure the mice were held in an acrylic plastic container constructed so that each mouse would be in a restricted area but with adequate air ventilation. Groups of 3 treated and 3 control animals from each inbred strain were sacrificed at 2, 3, 6, 9, and 12 months of age by CO_2 asphyxiation, and the mammary glands and reproductive tracts were isolated for further whole-mount and histologic analysis. The remaining animals were sacrificed at 24 months or when moribund, in accordance with NIEHS animal care and use guidelines. At the 24-month sacrifice, the mammary glands were dissected from the pelts and all

remaining tissues were prepared for histology. For mice sacrificed in moribund condition, the mammary glands were isolated, and the mice were subjected to a necropsy in which either only affected organs or all tissues were retained for histologic analysis. Mammary tumors were isolated and prepared for histology. If tumors were large enough to be divided, half was prepared for routine histology and the remainder was immediately frozen in liquid nitrogen.

Whole Mounts and Histology

Mammary ductal morphology was evaluated in whole mount preparations from untreated and radiation-exposed mice. Two methods were used to stain mammary glands. In the first method, the mammary glands were fixed on the pelts in 10% neutral buffered formalin for 18 to 24 h, dissected and placed into histocassettes, defatted with acetone, and stained with O-toludine blue, essentially as described by Russo (22). In the alternative staining method, the whole mammary glands were stained using carmine red (Sigma), essentially as described by Bannerjee et al. (23). The stained fourth abdominal mammary glands were mounted on 3-in x 2-in slides using Permount (Fisher Scientific) and a 35-mm x 50-mm glass cover slip. Mammary glands that had apparent preneoplastic or neoplastic nodules were selected for histology. The mounted glands were released from the Permount using xylenes and rinsed in 100% ethanol, rehydrated through graded alcohols, and processed for routine histology.

At terminal necropsy, all isolated tissues were fixed in 10% neutral buffered formalin for 24 hours, changed to 70% ethanol, processed for routine histology, and evaluated for pathology.

Mammary gland ductal morphology

The mammary glands from radiation-exposed and control mice were coded and graded for the extent of overall branching complexity on a scale of 1 (minimal complexity; simple) to 4 (maximal complexity; highly complex) as previously described (24). The criteria for grades included extent of growth into the fat pad, complexity of side-branching and degree of epithelial density, which were reflected in the relative number of terminal end buds, lateral buds, and/or alveolar buds penetrating the surrounding stroma. Differences between treatment groups were analyzed either by Wilcoxin Rank Sum or Mann-Whitney U test (25) as previously described (24).

Results

Mammary ductal morphogenesis

One major objective of this study was to characterize ductal branching patterns, in control and irradiated inbred mouse strains commonly used for carcinogenesis studies and the development of genetically engineered mouse models. Mammary ductal morphogenesis was highly mouse strain specific as characterized by the extent of overall branching complexity and the response to radiation. Average grades for branching complexity were determined for radiation-exposed and control inbred mouse strains (Table I). Whereas the combination of characteristics was unique for each inbred strain, some similarities were noted in irradiated and control mice. For all strains, proliferating terminal end buds were largely visible extending into the surrounding stroma at 2 months of age. By 3 months of age this process was largely complete and the ductal epithelium had reached the limits of the mammary fat pad. The SWR strain were exceptional in this respect since they retain a significant number of TEBs at three months of age compared to the other strains.

Mammary ductal morphology in BALB/c inbred mice

Mammary glands from BALB/c mice initially responded to radiation exposure with a mild inhibition of side-branching and alveolar bud formation. This effect was visible in treated mice at 3 and 6 months of age (Figure 1) and is reflected by the mammary gland complexity grades (Table I). By 9 months of age, nodules were apparent in the abdominal mammary glands isolated from 2 of the 3 treated animals and dilated ducts were visible in all radiation-treated mice (Figure 1C). At 12 months of age 2 of the 3 radiation-treated mice examined had severely dilated ducts filled with proteinaceous material (Figure 1E) and galactoceles (not shown). The difference in ductal branching morphology between control and radiation-treated mice were marked. The mammary glands isolated from 12 month-old radiation-exposed mice displayed increased complexity that was characterized by budding ductules and the formation of lobule-like structures. Additional radiation-induced histologic alterations included mild galactophoritis, glandular differentiation and minimal ductular and alveolar atypical hyperplasia.

Mammary ductal morphogenesis in FVB mice

In contrast to BALB/c, FVB mice responded to radiation exposure with a marked reduction of ductal branching and alveolar bud formation. This altered morphology was first apparent at 2 months of age and more dramatic differences between control and exposed mice were observed at 3 months of age (Figure 2). The average ductal morphology grades between control and irradiated mice examined at 6 months of age did not differ significantly. However significant differences did exist in mammary ductal branching patterns between treated and untreated mice examined at 9, 12 (Table I) and 15 month timepoints (Figure 2C and 2D).

Mammary ductal morphogenesis in C57BL/6 mice

Overall, the mammary ductal branching pattern was less complex in C57BL/6 mice than the other strains evaluated in this study (Figure 3). The ductal branching pattern did not differ between the control and radiation-exposed mice at all timepoints examined. At two months of age the ductal branching pattern was characterized by relatively few side-branches emanating from the long primary ducts. This branching pattern was remained essentially unchanged at every timepoint examined and no differences were observed between radiation treated and untreated mice (Table I).

Mammary ductal morphogenesis in SWR mice

At 2 and 3 months the mammary glands from control and irradiated SWR mice displayed elongated ducts with terminal end buds, but little side-branching or alveolar bud formation (data not shown). In contrast to other strains, TEBs were still present at 3 months and the mammary epithelium had not yet reached the limits of the fat pad (Figure 4). However, by six months of age, the ducts of both treated and control mice had numerous side-branches and were decorated liberally with alveolar buds. In general, cells were piled into disorganized layers around the lumen of the mammary gland. The morphology observed in the 6-month old mice persisted until the end of the 2-year study period for both treatment groups. Histological analysis revealed a dense pattern of epithelial cells lining the lumen of the mammary ducts at 9 months of age. The mammary glands of radiation-exposed mice were characterized by subtle increases in ductal and alveolar bud formation. However, no overall differences in average grade of ductal branching were observed between control and exposed mice at any timepoint examined (Table I).

Mammary ductal morphogenesis in C3H mice

A complex mammary ductal architecture was observed in the mammary glands of C3H mice as early as 3 months. At 2 months of age terminal end buds were visible (Figure 5A and 5B), side branches had formed, and alveolar buds were apparent on the elongated ducts in both radiation treated and untreated mice: However the complexity of branching was less in the radiation treated group. In contrast to the relatively simple branching pattern at 2 months, the mammary ducts of control and exposed mice were highly complex at 3 months of age and were characterized by numerous side branches and alveolar buds emanating from the ductal network (Figure 5C and 5D). Similar patterns were observed at the six month and later timepoints (Table I).

Radiation-induced tumorigenesis

Another objective of this study was to identify inbred mouse strains that were sensitive and resistant to organ-specific tumorigenesis. The five inbred mouse strains evaluated in this study differed in their susceptibility to radiation-induced and spontaneous mammary tumorigenesis. BALB/c and SWR mice were the most sensitive strains to radiation-induced mammary tumorigenesis, with tumor incidences of 27% and 58%, respectively (Table II). SWR mice were also the most sensitive to spontaneous mammary tumor development. In contrast, the C57BL/6, FVB, and C3H strains were relatively resistant to radiation-induced mammary tumorigenesis.

Mammary tumors were evaluated using the Annapolis criteria (26) and the primary histological patterns differed substantially between SWR and BALB/c mice. All but two of the mammary tumors from treated and control SWR mice were characterized as solid carcinomas

with fibrosis (Figure 6A). In contrast, radiation-treated BALB/c mice developed a diverse spectrum of mammary tumors that included three glandular (Figure 6B), three papillary (Figure 6C), two adenosquamous and one acinar carcinoma. Both tumors from untreated BALB/c mice were glandular carcinomas.

The five inbred mouse strains evaluated in this study differed in susceptibility to radiation-induced ovarian tumor development (Table II). The C3H strain was extremely susceptible to radiation-induced ovarian tumorigenesis with an incidence of 95% and this sensitivity was paralleled by a relatively high spontaneous ovarian tumor incidence of 20%. The remaining inbred strains were relatively resistant to spontaneous and radiation-induced ovarian tumorigenesis (Table II). The ovaries of radiation-exposed and control mice were compared histologically at the 3, 6, 9, and 12-month timepoints. In all cases mature follicles were present in both the radiation and control groups and the mice were ovulating. Radiation exposure did not inhibit the ovarian function that is required for ductal morphogenesis in the mammary gland. In general, radiation-exposed mice had fewer primary follicles than those of controls, which contributed to premature ovarian failure in all the inbred mouse strains examined in this study.

Lung and liver tumor development was evaluated in the animals that were sacrificed at the end of the 2-year period. Radiation-exposed C3H mice developed liver tumors with 59% incidence (10 of 17 examined) compared to 21% incidence (3 of 14 examined) for the controls. Lung tumors occurred in the radiation exposed SWR mice examined with an incidence of 100% (7 of 7 mice examined) and 38% (5 of 13 examined) in radiation-exposed BALB/c mice, compared to 50% (6 of 12 examined) and 24% (4 of 17 examined) for their untreated controls, respectively. These results are consistent with previous studies comparing chemically induced carcinogenesis in these inbred mouse strains (16). For example C3H mice are highly sensitive to

chemically induced hepatocarcinogenesis and SWR mice are among the most susceptible to chemically induced lung tumor development (16).

Long-term survival in radiation-exposed and control mice

Survival curves were generated for the radiation-exposed and control mice in each of the strains examined (Figure 7). Mice sacrificed at interim time points for the isolation of mammary glands were not included in this analysis. Overall, the radiation-exposed BALB/c and SWR strains displayed a reduction in time to tumors, and more specifically to mammary tumors, relative to the untreated controls. There was an initial decline in survival for the control and radiation-exposed FVB mice at 5 to 6 months of age. These deaths were likely the result of the well-documented lethal epileptic syndrome in FVB mice that may have been exacerbated by the radiation exposure (27). Irradiated C57BL/6 mice began developing dermatitis about 4 months earlier than the untreated controls. The severity of dermatitis required the sacrifice of many C57BL/6 mice before the 24-month timepoint. The overall trend in survival actually appeared slightly improved for C3H mice exposed to radiation compared to untreated controls in spite of the high ovarian tumor incidence in the treated animals. The basis for this observation has not been determined.

Discussion

We provide for the first time a comprehensive comparison of normal mammary gland morphogenesis among commonly used inbred mouse strains and the consequences of relatively low-dose radiation exposure on mammary gland development and tumorigenesis. Low-level radiation exposure dramatically alters mammary ductal branching morphology in the BALB/c

and FVB inbred mouse strains. Radiation exposure induces a high incidence of mammary tumors in SWR and BALB/c females and ovarian tumors in C3H mice. Radiation-induced inhibition of ductal branching was initially apparent at three months in both BALB/c and FVB mice and this phenotype persisted in treated FVB mice. However, the mammary ducts in the glands of BALB/c mice, in response to radiation, displayed increased branching beginning at 9 months with severely dilated ducts, lobule-like structures, and ductule proliferation apparent at 12 months. These radiation-induced alterations in ductal morphology in BALB/c mice were associated with susceptibility to radiation-induced mammary tumorigenesis. In contrast, the radiation-induced inhibition of ductal branching in the FVB mice did not result in increased susceptibility to mammary tumorigenesis. Although SWR mice are sensitive to radiation-induced mammary tumorigenesis, no differences in mammary ductal branching patterns were observed between treated and control mice. The relative susceptibilities of the inbred strains to radiation-induced mammary carcinogenesis were: SWR > BALB/c > FVB, C57BL/6, C3H. The high susceptibility of SWR and BALB/c mice to spontaneous and/or radiation-induced mammary tumorigenesis may make these strains valuable for the identification of modifier genes that influence mammary cancer risk. In addition, these mouse strains may be attractive to investigators interested in developing gene-targeted and transgenic mice on genetic backgrounds that are susceptible to radiation-induced changes in mammary ductal morphogenesis and tumor susceptibility.

A polymorphism in the DNA-dependent protein kinase catalytic subunit gene has been identified in BALB/c mice and is associated with a reduced DNA repair capacity and an increased susceptibility to mammary tumorigenesis (28,29). This genetic variation may contribute to the severe radiation-induced ductal dilation, galactocele formation, and proliferation observed in this strain. Radiation appears to act as a complete carcinogen for

mammary tumorigenesis in BALB/c mice. Neoplastic and non-neoplastic alterations in the mammary gland may be a consequence of the reduced DNA repair capacity of this strain. A more efficient DNA repair capacity of FVB inbred mice may explain how radiation inhibits ductal morphology without the associated damage months after exposure, as is evident in the BALB/c strain.

C3H mice are highly sensitive to radiation-induced ovarian tumorigenesis compared to the other inbred strains studied. The relative susceptibilities of the inbred strains to radiation-induced ovarian carcinogenesis was: C3H >> BALB/c, FVB, SWR, C57BL/6. The 0.3 Gy radiation dose used in this study did not inhibit ovulation in any of the mice examined and as a result would not be expected to interfere with mammary ductal morphogenesis. However, radiation exposure was associated with a depletion of primordial follicles and premature ovarian failure in all inbred strains. Oocytes are relatively resistant to radiation-induced damage at birth, maximally susceptible at approximately 21 days, and become more resistant again in the adult mouse (30,31). The premature loss of follicles from the ovary and altered hormonal feed back in combination with the C3H genetic background appears to contribute to the dramatic increase in ovarian tumors in this inbred strain (30,31).

Ovarian tumor susceptibility has been studied in several inbred mouse strains. For example, C3H mice are sensitive to both spontaneous and chemically induced ovarian tumors (31,32) whereas C57BL/6 and BALB/c mice are relatively resistant (31). Young SWR mice develop a low incidence of spontaneous granulosa cell tumors (33,34) and have been proposed as a model for a juvenile onset ovarian tumor syndrome in humans. Genetic mapping studies suggest this trait is controlled by up to four loci in crosses with SWR mice (35). Radiation

treated (BALB/c x C57BL/6)F1–Apc1638N developed ovarian tumors whereas F1s from crosses with other inbred strains did not (20).

Inbred mouse models with known responses to environmental exposures associated with human cancer risk are indespensable for examining the effects of age, reproductive history, timing of exposure, as well as strategies to prevent breast cancer. Strains demonstrating phenotypic extremes can be used to understand the impact of genetics in combination with environmental expousre that would be extremely difficult to detect in epidemiologic studies.

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References:

1. Goss, P. and Sierra S. (1998) Current Perspectives on Radiation-Induced Breast Cancer. *J Clin Oncol*, **16**, 338-347.
2. Hancock, S.L., Tucker M.A. and Hoppe R.T. (1993) Breast cancer after treatment of Hodgkin's disease. *J Natl Cancer Inst*, **85**, 25-31.
3. Howe, G. and McLaughlin J. (1996) Breast cancer mortality between 1950 and 1987 after exposure to fractionated moderate-dose-rate ionizing radiation in the Canadian

Fluoroscopy Cohort Study and a comparison with breast cancer mortality in the Atomic Bomb Survivors Study. *Radiat Res*, **145**, 694-707.

4. Hudson, M.M., Poquette C.A., Lee J., Greenwald C.A., Shah A., Luo X., Thompson E.I., Wilimas J.A., Kun L.E. and Crist W.M. (1998) Increased mortality after successful treatment for Hodgkin's disease. *J Clin Oncol*, **16**, 3592-3600.

5. Tokunaga, M. (1978) Breast cancer among atomic bomb survivors; relationship of prognosis to pathologic findings. *Radiation Effects Research Foundation*, **RERF TR 14-78**, 1-16.

6. Barcellos-Hoff, M.H. (1998) The Potential Influence of Radiation-Induced Microenvironments in Neoplastic Progression. *J Mamm Gland Bio Neoplasia*, **3**, 165-175.

7. Crompton, N.E.A. (1998) Programmed Cellular Response in Radiation Oncology. *ACTA Oncologica*, **37**, 1-49 Suppl. 11.

8. Hutchinson, F. (1998) Molecular biology of mutagenesis of mammalian cells by ionizing radiation. *Semin Cancer Biol*, **4**, 85-92.

9. Kastan, M.B., Onyekwere O., Sidransky D., Vogelstein B. and Craig R.W. (1991) Participation of p53 protein in the cellular response to DNA damage. *Cancer Res.*, **51**, 6304-6311.

10. Lane, D. (1998) Awakening angels. *Nature*, **394**, 616-617.

11. Storer, J.B. (1972) Acute Responses to Ionizing Radiation. In Green, E.L. (ed.), *Biology of the Laboratory Mouse*. Jackson Laboratory, New York, pp. 427-446.

12. Storer, J.B., Mitchell T.J. and Fry J.M. (1988) Extrapolation of the relative risk of radiogenic neoplasms across mouse strains and to man. *Radiat. Res.*, **114**, 331-353.

13. Roderick, T. (1963) The response of twenty-seven inbred strains of mice to daily doses of whole body X-irradiation. *Radiat Res*, **20**, 631-639.

14. Ullrich, R.L., Jernigan M.C., Satterfield L.C. and Bowles N.D. (1987) Radiation carcinogenesis: Time-dose relationships. *Radiat. Res.*, **111**, 179-184.

15. Dietrich, W.F., Lander E.S., Smith J.S., Moser A.R., Gould K.A., Luongo C., Borenstein N. and Dove W. (1993) Genetic identification of *Mom-1*, a Major Modifier Locus Affecting *Min*-induced intestinal neoplasia in the mouse. *Cell*, **75**, 631-639.

16. Drinkwater, N.R. and Bennett L.M. (1990) Genetic control of carcinogenesis in experimental animals. In Ito, N. (ed.), *Modification of Tumor Development in Rodents.* Karger A.G., Basel, pp. 1-20.

17. Bennett, L.M., Winkler M.L. and Drinkwater N.R. (1993) A gene that determines the high susceptibility of the C3H/HeJ strain of mouse to liver tumor induction is located on chromosome one. *Proc Am Assoc Cancer Res*, **33**, 164.

18. Lee, G.H., Bennett L.M., Carabeo R.A. and Drinkwater N.R. (1995) Identification of hepatocarcinogen-resistance genes in DBA/2 mice. *Genetics*, **139**, 387-95.

19. Moser, A.R., Hegge L.F. and Cardiff R.D. (2001) Genetic background affects susceptibility to mammary hyperplasias and carcinomas in Apc(min)/+ mice. *Cancer Res*, **61**, 3480-5.

20. van der Houven van Oordt, C., Smits R., Schouten T.G., Houwing-Duistermaat J.J., Williamson S.L.H., Luz A., Khan P.M., van der Eb A.J., Breuer M.L. and Fodde R. (1999) The genetic background modifiers the spontaneous and X-ray-induced tumor spectrum in the Apc1638N mouse model. *Genes Chromosomes Cancer*, **24**, 191-198.

21. Bennett, L.M. (1999) Breast cancer: Genetic predisposition and exposure to radiation. *Molecular Carc*, **26**, 143-149.

22. Russo, I.H. and Russo J. (1996) Mammary gland neoplasia in long-term rodent studies. *Environ Health Perspect*, **104**, 938-67.

23. Bannerjee, M.R., Wood B.G., Lin F.K. and Crump L.R. (1976) Organ culture of the whole mammary gland of the mouse. *Tissue Culture Association Manual*, **2**, 457-461.

24. Bennett, L.M., McAllister K.A., Malphurs J., Ward T., Collins N.K., Seely J.C., Gowen L.C., Koller B.H., Davis B.J. and Wiseman R.W. (2000) Mice heterozygous for a *Brca1* or *Brca2* mutation display distinct mammary gland ovarian phenotypes in response to diethylstilbestrol. *Cancer Res*, **60**, 3461-3469.

25. Siegel, S. (1956) *Nonparametric statistics for the behavioral sciences*. McGraw-Hill Book Company, New York.

26. Cardiff, R.D., Anver M.R., Gusterson B.A., Hennighausen L., Jensen R.A., Merino M.J., Rehm S., Russo J., Tavassoli F.A., Wakefield L.M., Ward J.M. and Green J.E. (2000) The mammary pathology of genetically engineered mice: the consensus report and recommendations from the Annapolis meeting. *Oncogene*, **19**, 968-88.

27. Goelz, M.F., Mahler J., Harry J., Myers P., Clark J., Thigpen J.E. and Forsythe D.B. (1998) Neuropathologic findings associated with seizures in FVB mice. *Lab Anim Sci*, **48**, 34-7.

28. Okayasu, R., Suetomi K., Yu Y., Silver A., Bedford J.S., Cox R. and Ullrich R.L. (2000) A deficiency in DNA repair and DNA-PKcs expression in the radiosensitive BALB/c mouse. *Cancer Res*, **60**, 4342-5.

29. Yu, Y., Okayasu R., Weil M.M., Silver A., McCarthy M., Zabriskie R., Long S., Cox R. and Ullrich R.L. (2001) Elevated breast cancer risk in irradiated BALB/c mice associates with unique functional polymorphism of the Prkdc (DNA-dependent protein kinase catalytic subunit) gene. *Cancer Res*, **61**, 1820-4.

30. Capen, C.C., Beamer W.G., Tennent B.J. and Stitzel K.A. (1995) Mechanisms of hormone-mediated carcinogenesis of the ovary in mice. *Mutat Res*, **333**, 143-51.

31. Marchant, J. (1977) Animal models for tumors of the ovary and uterus. In Cotchin, E. and Marchant, J. (eds), *Animal tumors of the female reproductive tract: spontaneous and experimental*. Springer-Verlag, New York, pp. 1-65.

32. Frith, C.H., Zuna R.E. and Morgan K. (1981) A morphologic classification and incidence of spontaneous ovarian neoplasms in three strains of mice. *J Natl Cancer Inst*, **67**, 693-702.

33. Fox, H. (1985) Sex cord-stromal tumours of the ovary. *J Pathol*, **145**, 127-48.

34. Beamer, W.G., Hoppe P.C. and Whitten W.K. (1985) Spontaneous malignant granulosa cell tumors in ovaries of young SWR mice. *Cancer Res*, **45**, 5575-81.

35. Beamer, W.G., Shultz K.L., Tennent B.J., Nadeau J.H., Churchill G.A. and Eicher E.M. (1998) Multigenic and imprinting control of ovarian granulosa cell tumorigenesis in mice. *Cancer Res*, **58**, 3694-9.

36. Ross, R., Paganini-Hill A., Wan P. and Pike M. (2000) Effect of hormone replacement therapy on breast cancer risk: estrogen versus estrogen plus progestin. *J Natl Cancer Inst*, **92**, 328-332.

37. Greendale, G.A., Reboussin B.A., Sie A., Singh H.R., Olson L.K., Gatewood O., Bassett L.W., Wasilauskas C., Bush T. and Barrett-Connor E. (1999) Effects of estrogen and

estrogen-progestin on mammographic parenchymal density. Postmenopausal Estrogen/Progestin Interventions (PEPI) Investigators. *Ann Intern Med*, **130**, 262-9.

38. Lundstrom, E., Wilczek B., von Palffy Z., Soderqvist G. and von Schoultz B. (1999) Mammographic breast density during hormone replacement therapy: differences according to treatment. *Am J Obstet Gynecol*, **181**, 348-52.

39. Hofseth, L., Raafat A., Osuch J., Pathak D., Slomski C. and Haslam S. (1999) Hormone replacement therapy with estrogen or estrogen plus medroxyprogesterone acetate is associated with increased epithelial proliferation in the normal postmenopausal breast. *J Clin Endocrinol Metab*, **84**, 4559-4565.

40. Byrne, C., Colditz G.A., Willett W.C., Speizer F.E., Pollak M. and Hankinson S.E. (2000) Plasma insulin-like growth factor (IGF) I, IGF-binding protein 3, and mammographic density. *Cancer Res*, **60**, 3744-8.

41. Atkinson, C., Warren R., Bingham S.A. and Day N.E. (1999) Mammographic patterns as a predictive biomarker of breast cancer risk: effect of tamoxifen. *Cancer Epidemiol Biomarkers Prev*, **8**, 863-6.

42. Sala, E., Warren R., McCann J., Duffy S., Luben R. and Day N. (2000) Smoking and high-risk mammographic parenchymal patterns: a case-control study. *Breast Cancer Res*, **2**, 59-63.

43. Rosen, C.J., Dimai H.P., Vereault D., Donahue L.R., Beamer W.G., Farley J., Linkhart S., Linkhart T., Mohan S. and Baylink D.J. (1997) Circulating and skeletal insulin-like growth factor-I (IGF-I) concentrations in two inbred strains of mice with different bone mineral densities. *Bone*, **21**, 217-23.

44. Kleinberg, D.L., Feldman M. and Ruan W. (2000) IGF-I: an essential factor in terminal end bud formation and ductal morphogenesis. *J Mammary Gland Biol Neoplasia*, **5**, 7-17.

45. Boyd, N.F., Martin L.J., Stone J., Greenberg C., Minkin S. and Yaffe M.J. (2001) Mammographic densities as a marker of human breast cancer risk and their use in chemoprevention. *Curr Oncol Rep*, **3**, 314-21.

Figure 1. Mammary ductal morphogenesis in radiation exposed and control BALB/c mice. Mammary glands isolated from: three month (A) treated and (B) control; nine month (C) treated and (D) control; and 12 month (E) treated and (F) control mice. Radiation exposed mice display nodules and some ductal dilation at 9 months and more severe ductal dilation by 12 months.

Figure 2. Mammary ductal morphogenesis in radiation exposed and control FVB mice. Mammary glands isolated from: three month (A) treated and (B) control mice and fifteen month (C) treated and (D) control mice. Radiation exposure results in the inhibition of complex branching patterns in FVB mice first evident at 3 months and persisting to 15 months of age.

Figure 3. Mammary ductal morphogenesis in radiation exposed and control C57BL/6 mice. Mammary glands isolated from: three month (A) treated and (B) control mice and fifteen month (C) treated and (D) control mice. The simple branching pattern observed at three months is also evident 12 months later.

Figure 4. Mammary ductal morphogenesis in radiation exposed and control SWR mice. Mammary glands isolated from: three month (A) treated and (B) control, and six month (C) treated and (D) control mice. At three months the ducts are still proliferating to fill out the fat pad as evidenced by the presence of terminal end buds. By six months of age the glands of treated and control mice have complex branching patterns.

Figure 5. Mammary ductal morphogenesis in radiation exposed and control C3H mice. Mammary glands isolated from: two month (A) treated and (B) control, and six month (C)

treated and (D) control mice. The transition from simple to complex branching structures in C3H mice occurs rapidly. At two months terminal end buds are still present and there is a subtle difference in branching between treated and control glands. At six months the branching patterns are complex and do not differ between control and treated mice.

Figure 6. Photomicrographs of mammary tumors from radiation exposed BALB/c and SWR mice. (A) Representative solid carcinoma with fibrosis from the mammary gland of a radiation exposed SWR mouse (50x). (B) Representative glandular (25x) and (C) papillary (50x) carcinomas from radiation exposed BALB/c mice.

Figure 7. Survival curves for radiation-exposed and control inbred mice. The percent survival for each strain was plotted over the two-year time-period of this study. Animals sacrificed at interim time points were not included in this analysis. (A) BALB/cJ, (B) C57BL/6, (C) SWR/J, (D) C3H/He, (E) FVB/N.

Table I. Average Mammary Gland Complexity Grade[a] for Radiation Treated and Untreated Inbred Mouse Strains

Strain	Age (Months)				
	2	3	6	9	12
BALB/c Control	1.4	2.7	3.3	2.6	3.0
BALB/c Irradiated	1.4	2.0*	2.3*	3.2	3.8*
FVB Control	1.5	3.2	2.6	3.0	2.5
FVB Irradiated	1.3	1.0*	2.6	2.3*	2.0*
C57BL/6 Control	1.0	1.0	1.0	1.0	1.7
C57BL/6 Irradiated	1.0	1.0	1.0	1.0	1.3
SWR Control	1.2	1.0	3.7	3.8	3.7
SWR Irradiated	1.4	1.7	4.0	3.7	3.7
C3H Control	2.3	3.2	3.2	3.3	3.4
C3H Irradiated	1.8*	3.2	2.8	3.0	3.2

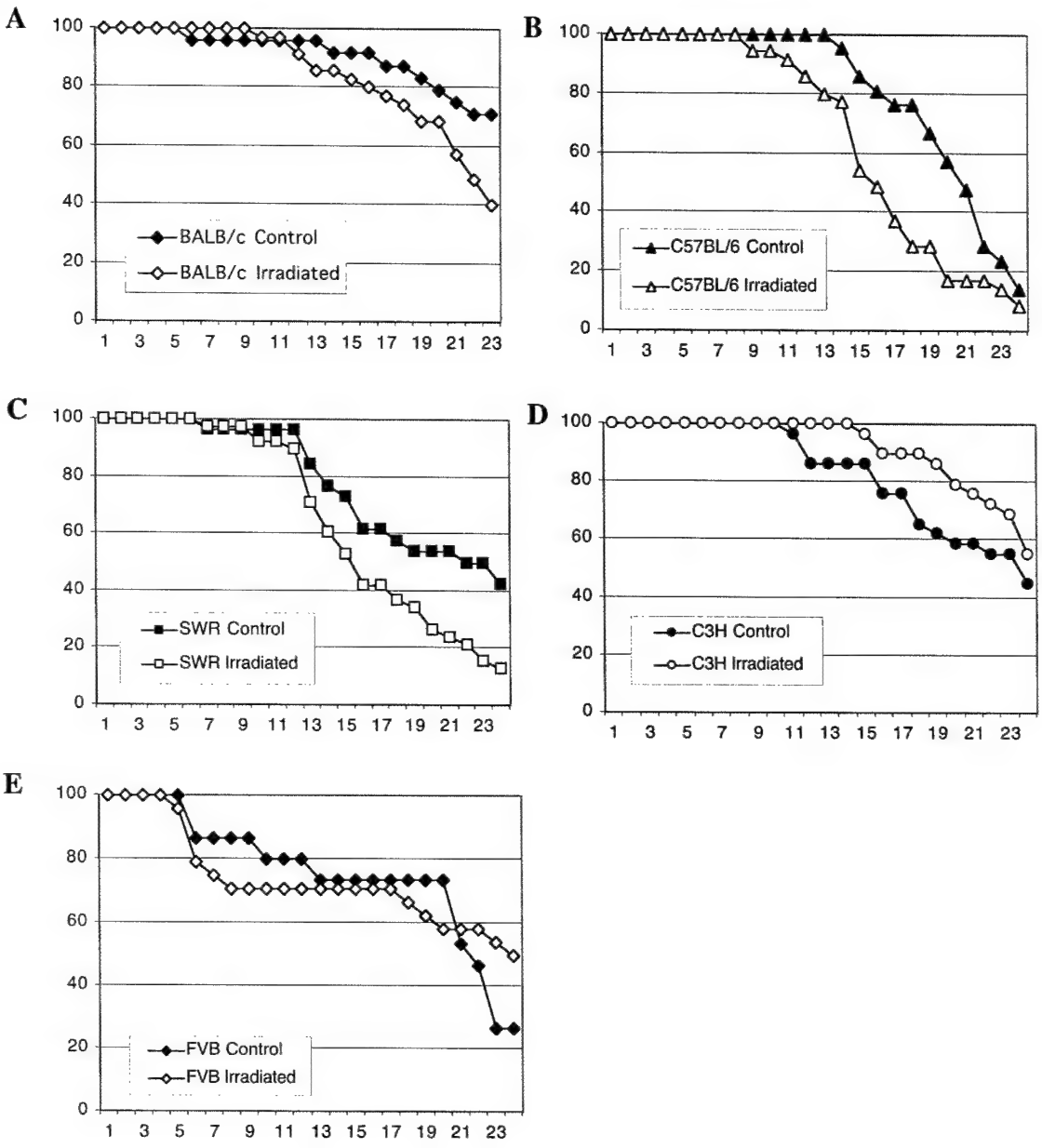
* statistically different from untreated control (Chi Square Analysis)

[a] See (24) and Materials and Methods

Table II. Mammary and ovarian tumor incidence in untreated and irradiated inbred mouse strains*

Strain	Mammary Tumors				Ovarian Tumors			
	Control	(%)	0.3 Gy	(%)	Control	(%)	0.3 Gy	(%)
BALB/c	2/25	(8.0)	9/34	(27)	0/30	(0)	4/36	(11)
SWR	7/23	(30)	19/33	(58)	0/19	(0)	1/25	(4.0)
C57BL/6	0/19	(0)	2/30	(6.6)	3/22	(14)	2/29	(6.9)
FVB	1/23	(4.4)	0/28	(0)	1/17	(5.9)	2/21	(9.5)
C3H	2/22	(9.1)	1/29	(3.4)	5/25	(20)	21/22	(95)

[a]Mammary tumors include adenosquamous carcinoma, adenocarcinoma, and carcinoma. Ovarian tumors include adenocarcinoma, adenomas, dysgerminoma, fibroma, granulosa cell tumor, luteoma, sertoli cell tumor and thecoma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BALB/c Control
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C57BL/6 Control
C57BL/6 Irradiated
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SWR Irradiated
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C3H Control
C3H Irradiated
E
FVB Control
FVB Irradiated

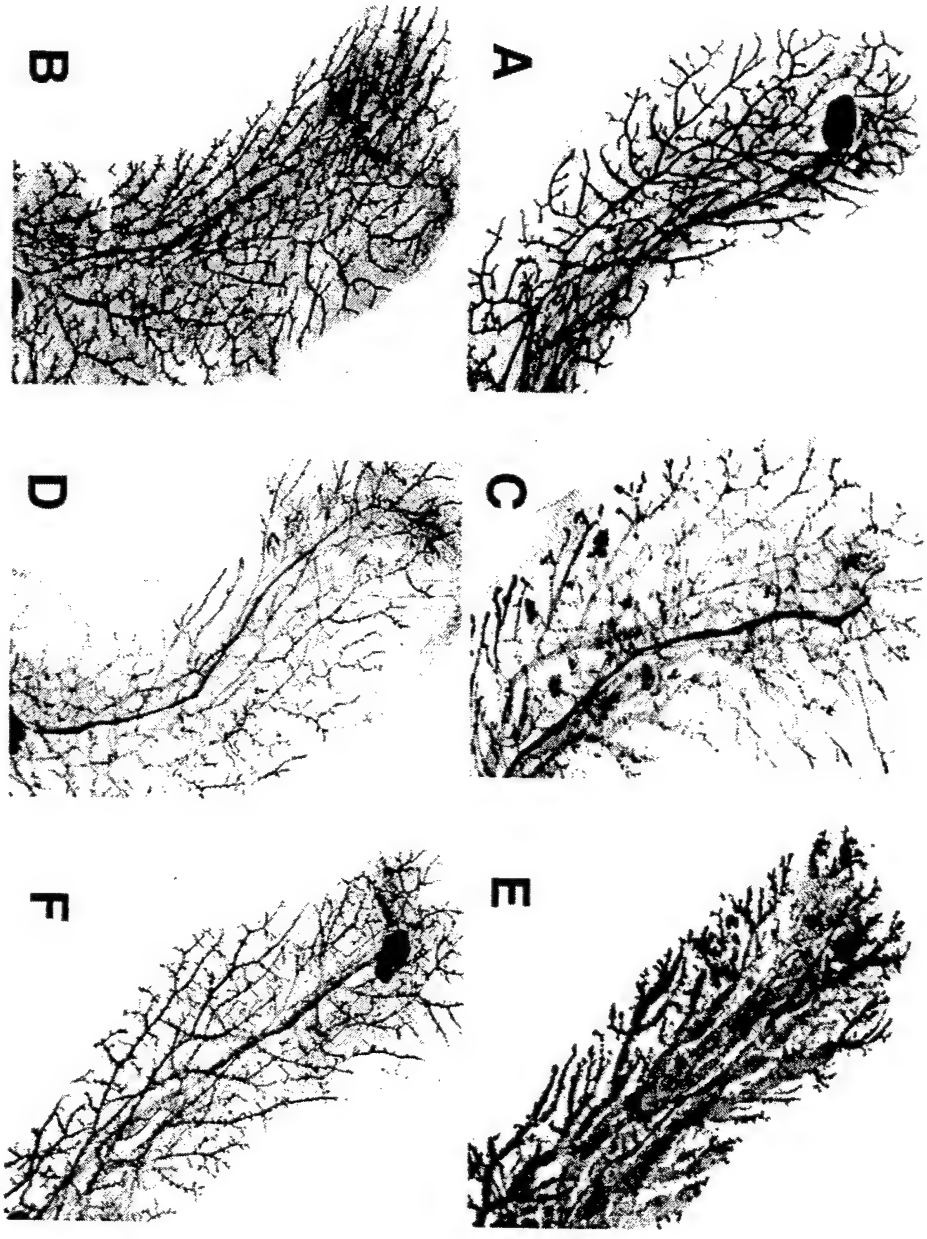

Figure 1

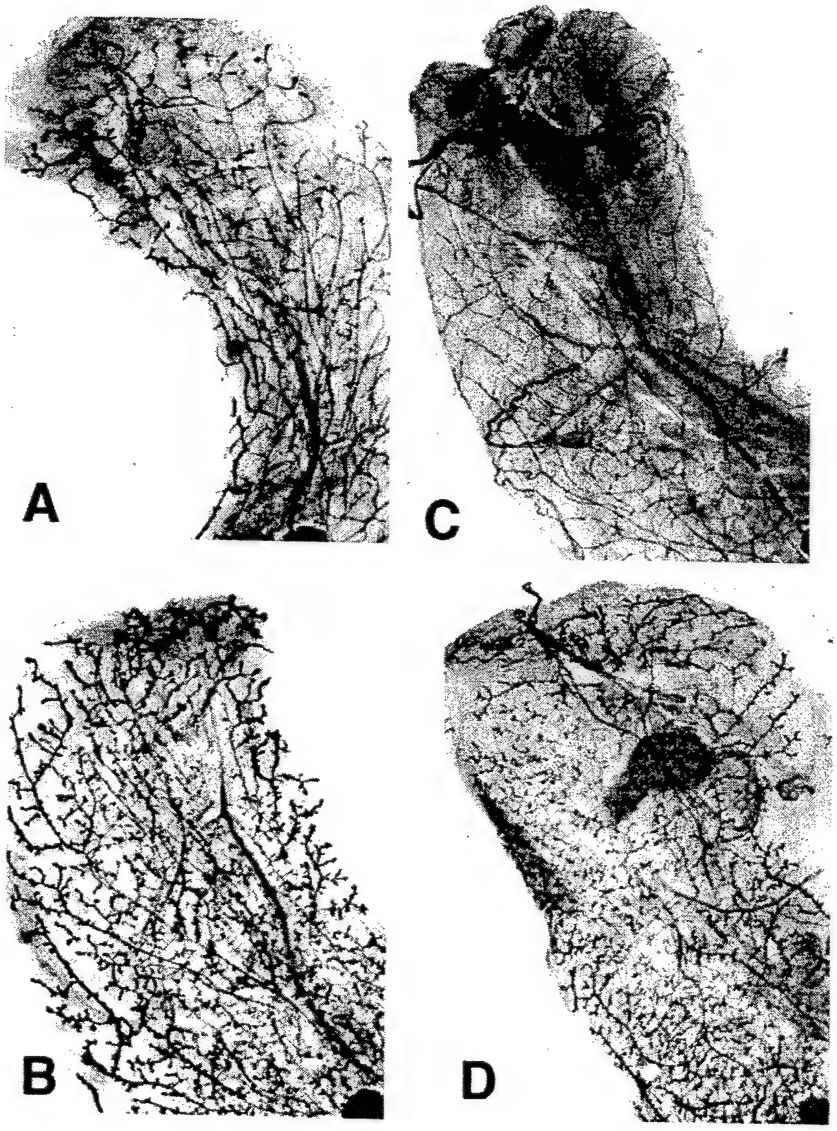

Figure 2

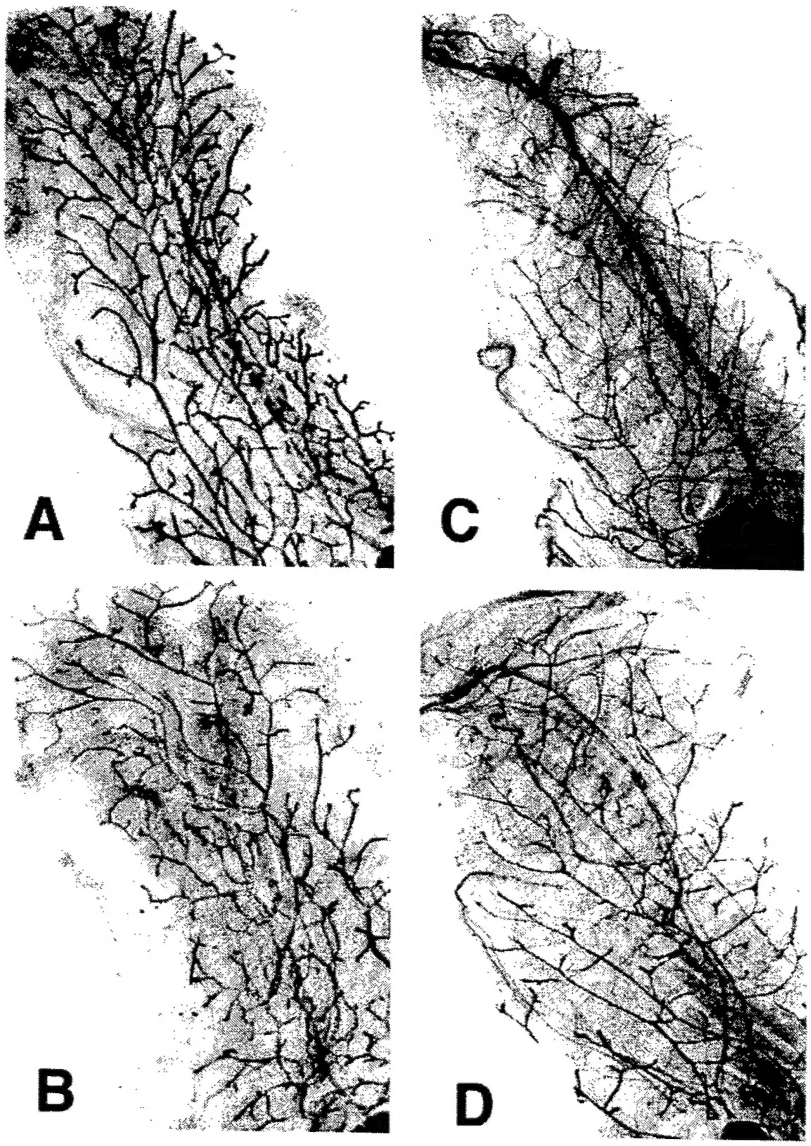

Figure 3

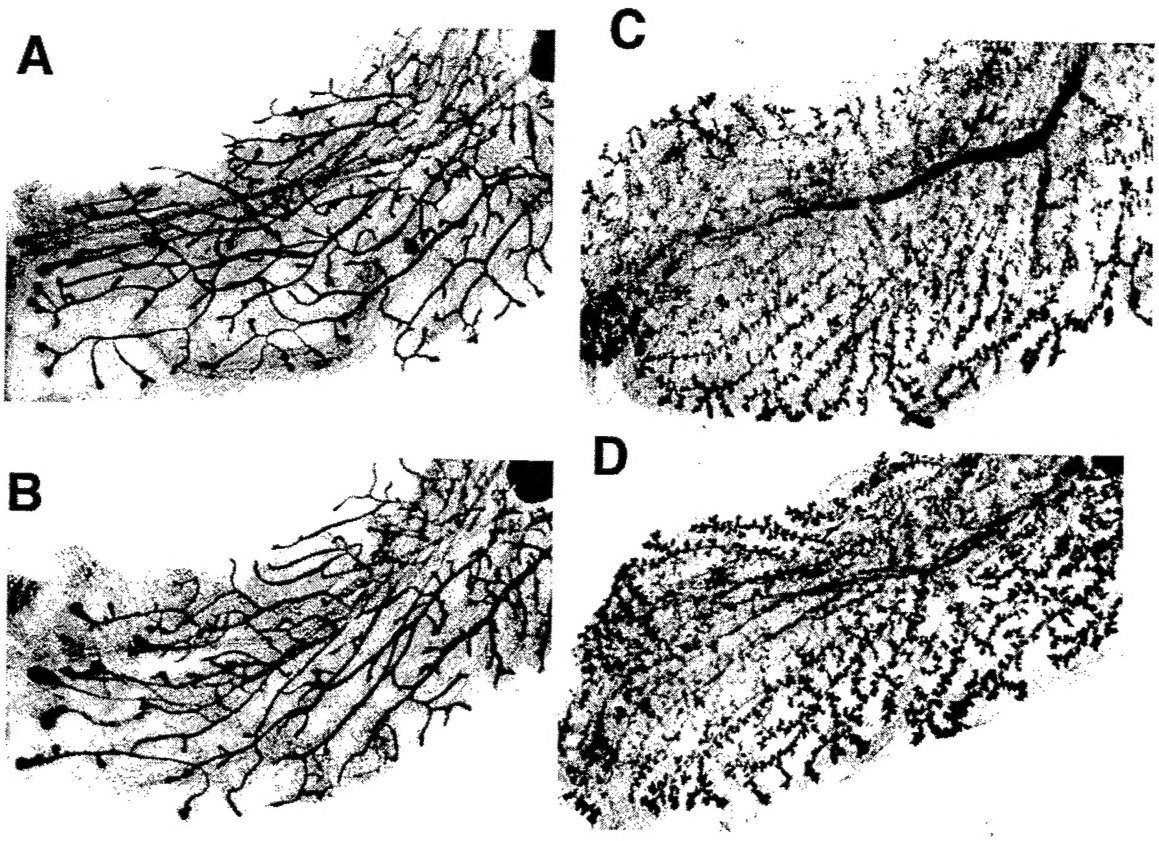

Figure 4

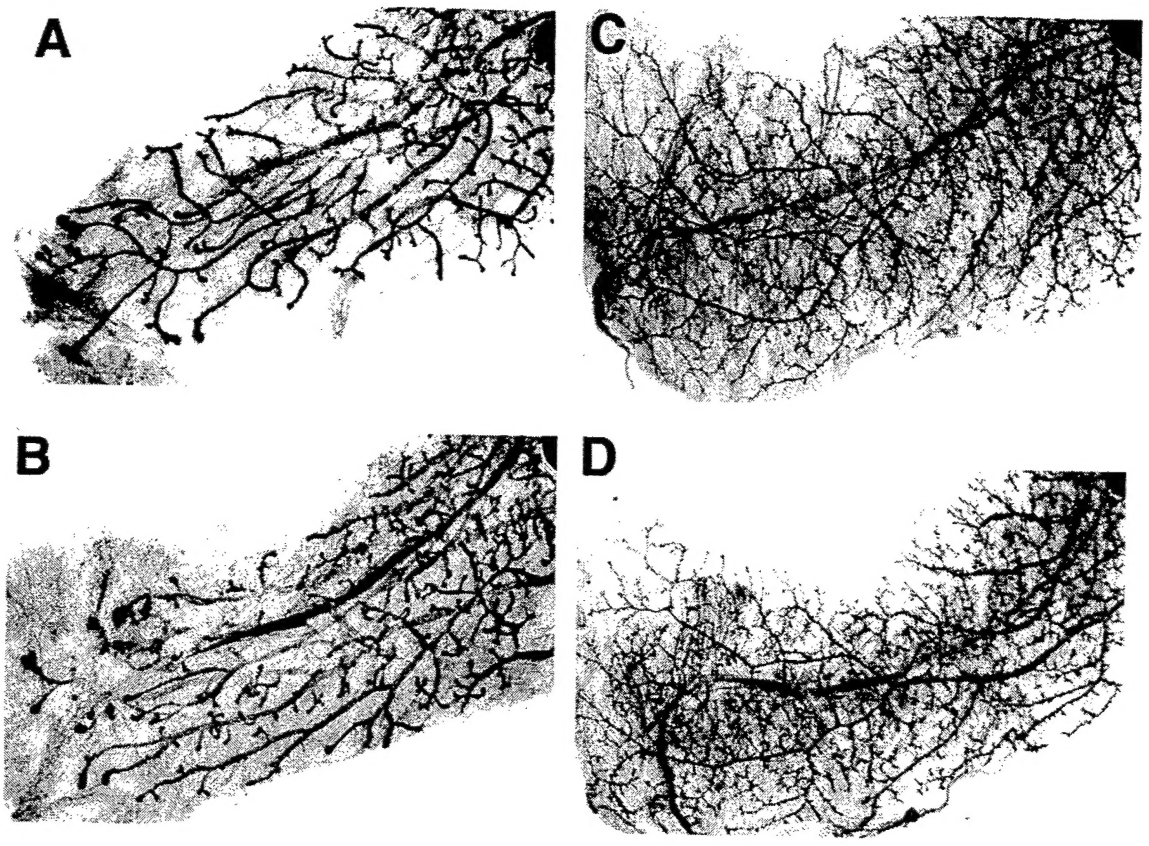

Figure 5

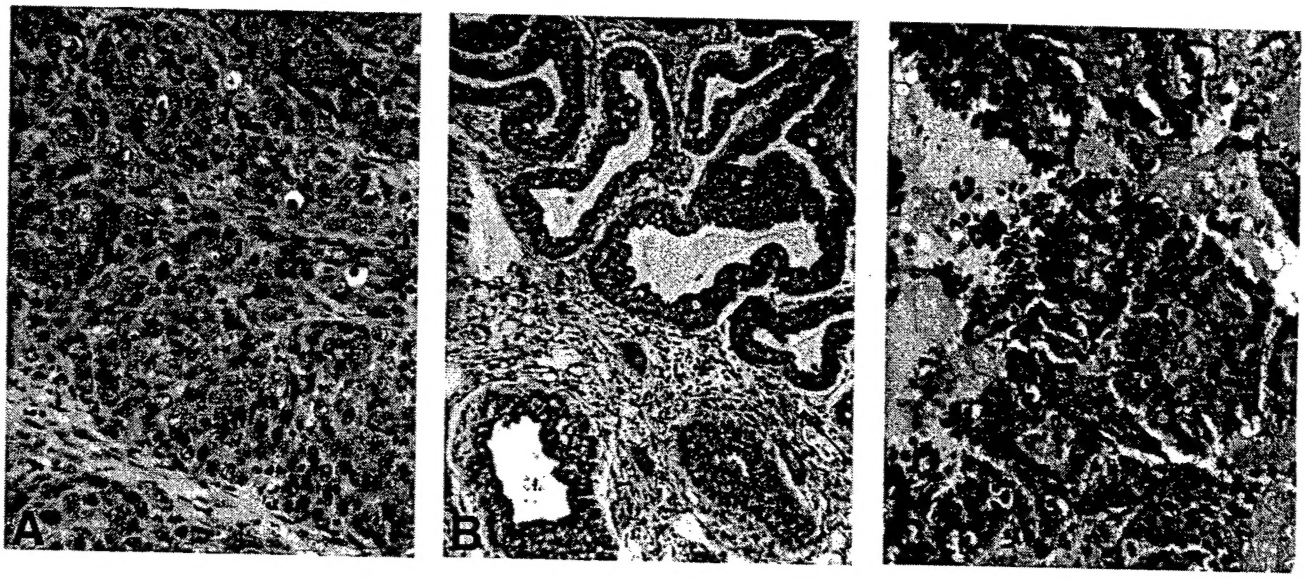

Figure 6